BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C07K 1/04, 17/06, 17/08 — A1

(11) International Publication Number: WO 94/11388

(43) International Publication Date: 26 May 1994 (26.05.94)

(21) International Application Number: PCT/AU93/00546

(22) International Filing Date: 25 October 1993 (25.10.93)

(30) Priority data:
07/972,755 6 November 1992 (06.11.92) US

(71) Applicant: CHIRON MIMOTOPES PTY. LTD. [AU/AU]; 11 Duerdin Street, Clayton, VIC 3168 (AU).

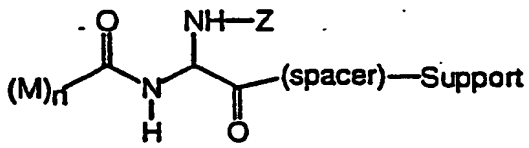
(72) Inventors: GEYSEN, H., Mario ; 31 Old Menzies Creek Road, Menzies Creek, VIC 3159 (AU). MAEJI, N., Joe ; 28 Adam Avenue, Hallam, VIC 3803 (AU). BRAY, Andrew, M. ; 48 Western Road, Boronia, VIC 3155 (AU). VALERIO, Robert, M. ; 17 Homestead Road, Cranbourne South, VIC 3977 (AU).

(74) Agents: SLATTERY, John, Michael et al.; Davies Collison Cave, 1 Little Collins Street, Melbourne, VIC 3000 (AU).

(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: SUPPORT FOR THE SYNTHESIS OF MODULAR POLYMERS



(I)

(57) Abstract

A support for the synthesis of multiple sets of several modular polymers, including peptides, consists of generally annular components with active surfaces, held co-axially by a retaining rod. Also disclosed is a bound molecular polymer of formula (I) wherein each M is independently a monomer; n is an integer from 2 to 100; and Z is a protecting group.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgystan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SI	Slovenia
CI	Côte d'Ivoire	KZ	Kazakhstan	SK	Slovakia
CM	Cameroon	LI	Liechtenstein	SN	Senegal
CN	China	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	LV	Latvia	TJ	Tajikistan
DE	Germany	MC	Monaco	TT	Trinidad and Tobago
DK	Denmark	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali	UZ	Uzbekistan
FR	France	MN	Mongolia	VN	Viet Nam
GA	Gabon				

SUPPORT FOR THE SYNTHESIS OF MODULAR POLYMERS

Technical Field

This invention relates to a solid surface for the synthesis of peptides thereon, and in particular relates to a rod or pin having a plurality of surfaces on which peptides may be synthesized and assayed efficiently and economically.

Background of the Invention

Geysen, EP 198855, disclosed a method for the simultaneous synthesis of a large number of different peptides. Basically, this method involves the synthesis of peptides on a solid polymeric surface, such as polyethylene, which may be molded into the shape of a rod or pin. In a preferred embodiment of the method, these rods or pins are positioned in a holder so that they form a 12 by 8 matrix, with the rods or pins being positioned so that the spacing corresponds to that of the wells of microtiter plates which are widely used for ELISA (enzyme-linked immunosorbent assay) tests.

The method disclosed in this prior specification was based on the realization that for the solid-phase synthesis of any peptide, almost all of the steps of the synthesis are exactly the same for any peptide and are independent of the sequence of the peptide being synthesized. Thus, with the rods or pins arranged in the preferred format so that 96 are in a holder, all steps in the synthesis such as washing steps, neutralization steps and deprotection steps can be carried out simultaneously in the synthesis of 96 different peptides. The only steps which must be carried out separately for each different peptide are the coupling of the appropriate amino acid residues. Each of these steps is conveniently carried out by dispensing appropriate

activated amino acid solution into the corresponding wells of a microtiter plate or the like. Thus, the appropriate amino acid is coupled to the peptide for each of the rods.

The quantity of peptide covalently bonded to the solid polymer surface by this method is sufficient to allow reaction of the peptide with specific binding entities such as antibodies to be readily detected. Although the quantity of peptide synthesized on each rod is relatively small (typically less than 1 μmole), the ability to reuse the synthesized peptide after a test compensates for the small quantity of peptide on the rod. However, in some applications the quantity of peptide needs to be greater. Examples of such applications include the removal of the peptide from the rod and recovery of specific binding entities for further testing. Modifications to the process of synthesis and testing of the peptides were disclosed in PCT WO91/04266.

It is an object of the present invention to provide means by which the amount of peptide, or for that matter any other polymeric compound such as nucleic acid which can be synthesized on a solid support, can be increased while retaining the advantage of being able to synthesize many peptides with different sequences simultaneously.

In the earlier method polyacrylic acid was grafted to the surface of a solid polyethylene support using gamma-irradiation. In that earlier system, the region on the rod on which peptide was grafted was completely defined by the volume of reagent used for the coupling of specific amino acids to the growing peptide, or more accurately, the depth into which the rod dipped into the amino acid solutions. As this depth inevitably varied slightly from cycle to cycle in the synthesis, the result was a small region on the rod where the peptide synthesized may have had appreciable amounts of deletion peptides (that is, peptides whose sequence varied from that intended by having one or more residues absent) present because slightly less of one of the activated amino acid solutions was dispensed in one or more cycles.

A further disadvantage of the earlier system is that the polymer layer grafted onto the rods during the radiation process is readily solvated by many sol-

vents and as a consequence, solvents will migrate upwards through this layer by capillary action. This results in depletion of the reservoir of reagent and consequently, as described above, a larger zone of uncertain synthesis quality is created. In addition, unless extreme care is taken in extensively washing these rods, the polymer layer acts as a reservoir of the solvent used in synthesis leading to the contamination of subsequent solutions.

Disclosure of the Invention

One aspect of the invention is an improved synthesis support, having a plurality of independent synthesis surfaces, which permits the simultaneous syntheses of peptides (or other modular polymers) having different N- or C-groups, having different linkages to the support (*e.g.*, permanent links, cleavable links, *etc.*), or otherwise differing in features other than sequence. Additionally, one can employ the new supports to make multiple copies of a collection of polymers.

Another aspect of the invention is a cleavable link which enables one to cleave modular polymers from the synthesis supports of the invention, leaving the modular polymer with an amide at the cleaved end.

Summary of the Drawings

Figure 1 depicts plan and cross-section views of an active surface component of the invention.

Figure 2 depicts plan views of support rod of the invention.

Figure 3 depicts ELISA results obtained using multiple epitope libraries prepared by the method of the invention.

Figure 4 depicts ELISA binding inhibition results obtained using multiple epitope libraries prepared by the method of the invention.

Modes of Carrying Out The Invention

A. Definitions

The term "modular polymer" refers to a polymer composed of non-identical subunits selected from a group of monomers. Modular polymers are generally synthesized one monomer at a time.

The term "monomer" as used herein refers to a molecule which may be coupled or condensed to form an oligomer. To provide diversity, monomers are selected from sets which contain at least four distinct members. Suitable monomer sets include conventional D- and L-amino acids, nucleic acids/nucleotides, carbohydrates, nonconventional D- and L-amino acids (*e.g.*, cyclohexylalanine, benzhydrylglycine, chloroalanine, and the like), and "peptoids" as described in WO91/19735 (incorporated herein by reference).

The term "conventional amino acid" refers to the amino acids alanine (A), cysteine (C), aspartic acid (D), glutamic acid (E), phenylalanine (F), glycine (G), histidine (H), isoleucine (I), lysine (K), leucine (L), methionine (M), asparagine (N), proline (P), glutamine (Q), arginine (R), serine (S), threonine (T), valine (V), tryptophan (W), and tyrosine (Y).

The term "nonconventional amino acid" refers to amino acids other than conventional amino acids. Presently preferred nonconventional amino acids are

Nle = L-norleucine;
Hphe = L-homophenylalanine;
Gabu = γ-aminobutyric acid;
Dcys = D-cysteine;
Dglu = D-glutamic acid;
Dhis = D-histidine;
Dlys = D-lysine;
Dmet = D-methionine;
Dpro = D-proline;
Darg = D-arginine;
Dthr = D-threonine;
Dtrp = D-tryptophan;
Dorn = D-ornithine;
Etg = L-ethylglycine;
Aabu = α-aminobutyric acid;
Nva = L-norvaline;
Dala = D-alanine;
Dasp = D-aspartic acid;
Dphe = D-phenylalanine;
Dile = D-isoleucine;
Dleu = D-leucine;
Dasn = D-asparagine;
Dgln = D-glutamine;
Dser = D-serine;
Dval = D-valine;
Dtyr = D-tyrosine;
Aib = aminoisobutyric acid;
Tbug = L-*t*-butylglycine;

Pen = penicillamine:
Chexa = cyclohexylalanine:
Cpro = aminocyclopropane carboxylate:
Mala = L-α-methylalanine:
Masp = L-α-methylaspartic acid:
Mphe = L-α-methylphenylalanine:
Mile = L-α-methylisoleucine:
Mleu = L-α-methylleucine:
Masn = L-α-methylasparagine:
Mgln = L-α-methylglutamine:
Mser = L-α-methylserine:
Mval = L-α-methylvaline:
Mtyr = L-α-methyltyrosine:
Mnle = L-α-methylnorleucine:
Mnva = L-α-methylnorvaline:
Metg = L-α-methylethylglycine:
Maib = α-methylaminoisobutyric acid:
Mpen = α-methylpenicillamine:
Mchexa = α-methylcyclohexylalanine:
Dmala = D-α-methylalanine:
Dmcys = D-α-methylcysteine:
Dmglu = D-α-methylglutamic acid:
Dmhis = D-α-methylhistidine:
Dmlys = D-α-methyllysine:
Dmmet = D-α-methylmethionine:
Dmpro = D-α-methylproline:
Dmarg = D-α-methylarginine:
Dmthr = D-α-methylthreonine:
Dmtrp = D-α-methyltryptophan:
Nmala = L-N-methylalanine:
Nmasp = L-N-methylaspartic acid:
Nmphe = L-N-methylphenylalanine:
Nmile = L-N-methylisoleucine:
Nmleu = L-N-methylleucine:
Nmasn = L-N-methylasparagine:
Nmgln = L-N-methylglutamine:
Nmser = L-N-methylserine:
Nmval = L-N-methylvaline:
Nmtyr = L-N-methyltyrosine:
Nmnle = L-N-methylnorleucine:
Nmnva = L-N-methylnorvaline:
Nmetg = L-N-methylethylglycine:
Nmcpen = N-methylcyclopentylalanine:

Anap = α-naphthylalanine;
Cpen = cyclopentylalanine;
Norb = aminonorbornylcarboxylate;
Mcys = L-α-methylcysteine;
Mglu = L-α-methylglutamic acid;
Mhis = L-α-methylhistidine:
Mlys = L-α-methyllysine;
Mmet = L-α-methylmethionine;
Mpro = L-α-methylproline;
Marg = L-α-methylarginine;
Mthr = L-α-methylthreonine;
Mtrp = L-α-methyltryptophan;
Morn = L-α-methylornithine;
Maabu = α-amino-α-methylbutyric acid;
Mhphe = L-α-methylhomophenylalanine;
Mgabu = α-methyl-γ-aminobutyric acid;
Mtbug = L-α-methyl-*t*-butylglycine;
Manap = α-methyl-α-naphthylalanine;
Mcpen = α-methylcyclopentylalanine;
Dmorn = D-α-methylornithine;
Dmasp = D-α-methylaspartic acid;
Dmphe = D-α-methylphenylalanine;
Dmile = D-α-methylisoleucine;
Dmleu = D-α-methylleucine;
Dmasn = D-α-methylasparagine;
Dmgln = D-α-methylglutamine;
Dmser = D-α-methylserine;
Dmval = D-α-methylvaline;
Dmtyr = D-α-methyltyrosine;
Nmcys = L-N-methylcysteine;
Nmglu = L-N-methylglutamic acid;
Nmhis = L-N-methylhistidine;
Nmlys = L-N-methyllysine;
Nmmet = L-N-methylmethionine;
Nmchexa = N-methylcyclohexylalanine;
Nmarg = L-N-methylarginine;
Nmthr = L-N-methylthreonine;
Nmtrp = L-N-methyltryptophan;
Nmorn = L-N-methylornithine;
Nmaabu = N-amino-α-methylbutyric acid;
Nmhphe = L-N-methylhomophenylalanine;
Nmgabu = N-methyl-γ-aminobutyric acid;
Nmtbug = L-N-methyl-*t*-butylglycine;

Nmpen = N-methylpenicillamine:
Nmaib = N-methylaminoisobutyric acid:
Dnmala = D-N-methylalanine:
Dnmcys = D-N-methylcysteine:
Dnmglu = D-N-methylglutamic acid:
Dnmhis = D-N-methylhistidine:
Dnmlys = D-N-methyllysine:
Dnmmet = D-N-methylmethionine:
Dnmpro = D-N-methylproline:
Dnmarg = D-N-methylarginine:
Dnmthr = D-N-methylthreonine:
Dnmtrp = D-N-methyltryptophan:
Nala = N-methylglycine (sarcosine):
Nglu = N-(2-carboxyethyl)glycine:
Nhhis = N-(imidazolylethyl)glycine:
Nlys = N-(4-aminobutyl)glycine:
Nmet = N-(2-methylthioethyl)glycine:
Nasn = N-(carbamylmethyl)glycine:
Nval = N-(1-methylethyl)glycine:
Nhtrp = N-(3-indolylethyl)glycine:
Nthr = N-(1-hydroxyethyl)glycine:
Norn = N-(3-aminopropyl)glycine:
Ncbut = N-cyclobutylglycine:
Nchep = N-cycloheptylglycine:
Ncdec = N-cyclodecylglycine:
Ncdod = N-cyclododecylglycine:
Nmanap = N-methyl-α-naphthylalanine:
Dnmorn = D-N-methylornithine:
Dnmasp = D-N-methylaspartic acid:
Dnmphe = D-N-methylphenylalanine:
Dnmile = D-N-methylisoleucine:
Dnmleu = D-N-methylleucine:
Dnmasn = D-N-methylasparagine:
Dnmgln = D-N-methylglutamine:
Dnmser = D-N-methylserine:
Dnmval = D-N-methylvaline:
Dnmtyr = D-N-methyltyrosine:
Nasp = N-(carboxymethyl)glycine:
Nphe = N-benzylglycine:
Nile = N-(1-methylpropyl)glycine:
Nleu = N-(2-methylpropyl)glycine:
Nhser = N-(hydroxyethyl)glycine:
Ngln = N-(2-carbamylethyl)glycine:
Narg = N-(3-guanidinopropyl)glycine:
Nhtyr = N-(p-hydroxyphenethyl)glycine:
Ncys = N-(thiomethyl)glycine: and
Ncpro = N-cyclopropylglycine:
Nchex = N-cyclohexylglycine:
Ncoct = N-cyclooctylglycine:
Ncund = N-cycloundecylglycine:
Nbhm = N-(2,2-diphenylethyl)glycine:
Nbhe = N-(3,3-diphenylpropyl)glycine:
Nnbhm = N-(N-(2,2-diphenylethyl)carbamylmethyl)glycine:
Nnbhe = N-(N-(3,3-diphenylpropyl)carbamylmethyl)glycine:
Nbmc = 1-carboxy-1-(2,2-diphenylethylamino)cyclopropane: and
Naeg = N-(2-aminoethyl)glycine.

The term "active surface" means a surface which is derivatized or otherwise rendered suitable for synthesis of modular polymers. It is "adapted" for use in the synthesis of modular polymers if modular polymers can be efficiently synthesized thereon. The component which carries the active surface may be homogeneous or heterogeneous in composition. For example, the active surface may be bonded or grafted to a supporting structure or surface. In principle, the active surface may be radiation grafted to any supporting structure (which may be, for example, an "inert surface" as defined below).

The term "inert surface" refers to a surface which is stable to the modular polymer synthesis conditions, and does not react. Suitable inert surfaces include without limitation polyethylene, polyolefins, cellulose acetate, wool, cotton, chitin, and the like.

B. General Method

In a first embodiment of the present invention, there is provided a plurality of supports for use in the synthesis of peptides or other polymeric compounds thereon, which supports comprise an inert surface and a set of active surfaces each comprising an active region on which said synthesis may take place.

The inert surface is typically provided in the form of a rod or pin of generally cylindrical shape, having space for a plurality of active surface components. Preferably, the inert support is capable of retaining 2 to 20 active surface components, more preferably about 5 active surface components. The support may have a cross-section that is circular, rectangular, or any other shape: circular and square are preferred. The support may further be provided with projections or protrusions to assist in placing, locating and retaining the active surface components. Further, the support may be provided with indentations and/or incisions to impart sufficient flexibility to the support that the active surface components may be "snapped" on. Alternatively, the active surface components may be positioned on a nail-shaped pin, followed by mounting the pin in a supporting array, where the "head" of the pin is shaped to retain all active surface components (the active surface components may be later removed by either removing the pin from its support, or by cleaving or removing the head of the pin).

The support rods may be grouped in an array to facilitate parallel processing, both during synthesis of the modular polymers and during their assay. In a presently preferred embodiment, the supports ("pins") are mounted in an 8 × 12 array on a block which matches the spacing of wells in a microwell assay plate. Preferably the rods are mounted by press-fit or friction-fit into holes drilled or molded in the

support block. The rods may include retaining flanges or projections to insure that each rod projects the same distance from the block surface. Alternative mounting means include threading the rods and holes, adhesives, one-piece molding, magnetic coupling, and the like. Other array formats are also considered within the scope of this invention. For example, the support rods may be positioned on a continuous belt, or may be gripped individually by robotic manipulators, to simplify automated handling.

Preferably, the active surfaces are provided in a form which may easily be attached to (and removed from) the inert support by friction fitting or snapping into place. However, other forms of attachment may be used, such as adhesive (which may or may not be permanent), heat fusing, threading (*e.g.*, like a nut and bolt), Velcro®, slot and key, and magnetism. For example, one may employ inert supports and active surface components having ferrous cores, and retain the active surface components by electromagnetism. In this embodiment, coupling reactions to the active surfaces may be accelerated by modulating the electric current, thereby inducing vibration of the pins. Effective vibration frequencies range from about 40 Hz to 60 KHz or more, preferably about 50-60 Hz. Alternatively, the supporting rods may be fashioned from, or affixed to, piezoelectric transducers which vibrate at the desired frequency when activated. The active surface components are preferably annular in shape, but may be formed in any shape which can be retained on the inert support. For example, active surface components may be semiannular (*e.g.*, completing about 270°) and still be retained by press-fit methods. Active surface components which are retained by magnetism need not be completely annular. The active surface components are preferably shaped to increase their surface area, so that they have a greater surface area than a cylinder of the same diameter. In one embodiment of the invention the active surface components are vaned or "gear" shaped, in order to maximize their surface area. The inner surface (facing the inert support) may be provided with flanges, ledges and/or other surfaces which may engage corresponding surfaces on the inert support. The terminal active surface component may have a different

shape, because the inert support need not pass entirely through the component. For example, the terminal active surface component may be hemispherical.

Figure 1 illustrates one presently preferred embodiment of the active surface component (*1*). Fig. 1A depicts a plan view of the component, having a thickness *t* of 2.5 mm, a maximum outer diameter of 5.5 mm, a minimum internal diameter (between opposed internal projections (*2*)) of 2.0 mm, with a maximum internal diameter of 3.0 mm. Figure 1B depicts a cross section of the component. The component is molded from polyethylene with 16 external "teeth" (*3*) and 4 internal teeth (*2*) arranged symmetrically.

Figure 2 illustrates one presently preferred embodiment of the support rod (*10*), designed for use with the components (*1*) of Fig. 1. Rod *10* comprises a primary shaft having a diameter of about 3.8 mm, and a length of about 32.3 mm. End *12* is rounded to facilitate insertion into the support block, and is provided with flange *13* to insure uniform insertion. Flange *13* is positioned about 9.8 mm from end *12*, and measures 1.1 mm in thickness by 6.0 mm in outer diameter. The secondary shaft (*14*) is coaxial with primary shaft *11*, and extends about 21.0 mm therefrom. Secondary shaft *14* has a diameter of about 2.0 mm, and is provided with a plurality of projections *15* of generally cylindrical shape, having a diameter of about 0.5 mm and extend about 0.85 mm from the surface of the secondary shaft *14*. These projections *15* serve to locate and position components *1* on secondary shaft *14*. Secondary shaft *14* is further provided with a generally frustroconical end cap *16* having a minimum diameter of about 1.0 mm and a maximum diameter of about 2.4 mm, sloping at about 30°. End cap *16* facilitates addition of the components *1* to the support *10*, and is responsible for retaining the bottom-most component.

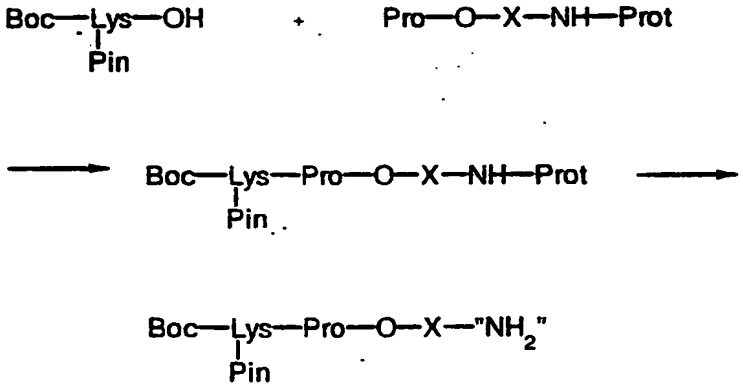
The active surfaces may be of identical or different compositions, depending upon the chemistry to which they will be subjected. The coating can be made of any of the porous resins which are used for conventional solid phase peptide and/or nucleic acid synthesis. Because these resins are porous, the surface area of the active region is increased dramatically and so allows a much greater yield of

modular polymer. In addition, the use of this embodiment of the invention makes it particularly convenient to change the chemistry used in the synthesis, and indeed, change the class of polymeric compounds to be synthesized by selecting the appropriate resin with which to coat the active surface component. Examples of porous resins which may be used in such coatings include benzhydrylamine-polystyrene resin and polyacrylamide gel inside kieselguhr. Other suitable surface materials include, without limitation, polyethylene glycol, cellulose and other natural polymers, Merrifield resin, Rink resin and polymers of acrylic acid, methylacrylate, methacrylic acid, methyl methacrylate, dimethylacrylamide, styrene, hydroxyethylacrylate, hydroxyethylmethacrylate, hydroxypropylmethacrylate, hydroxyethylmethacrylamide, methylmethacrylate, and polyethyleneglycol monomethacrylate, and the like, and combinations thereof. The component bearing the active surface need not be homogeneous. It is presently preferred to employ structural supports fashioned from not just polyethylene, polypropylene and its copolymers but also Teflon® (polytetrafluoroethylene) or any other stable inert surface. The active surface may then be attached to the supporting surface by any available means, including sintering, adhesive, heat fusing, and the like. The presently preferred method is grafting, by placing the support surface in a solution of solvent and active surface material and irradiating the mixture with gamma radiation. Preferred solvents are water, methanol (MeOH), H_2O/MeOH mixtures, dimethylformamide (DMF), and dimethylsulfoxide (DMSO). The mixture is prepared and irradiated as described in EP 138855: see also D. Müller-Schulte *et al.*, Polymer Bull (1982) 7:77-81.

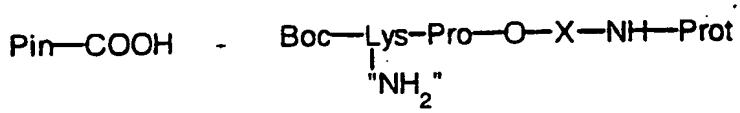
The surfaces are then modified, if desired, for the selected coupling chemistry. The modular polymers may be "permanent" (*i.e.*, not easily removed from the surface) or "cleavable" (designed for facile cleavage and removal from the support). Cleavable modular polymers will generally have a linkage to the active surface which facilitates cleavage from the surface under conditions not experienced during synthesis of the modular polymer. Different linking chemistries may also provide for different N-terminal and C-terminal groups (in the case of peptides). Cleavable mod-

ular polymers may be provided with labeling groups for detection, or binding ligands for purposes of separation and purification. For example, cleavable modular polymers may be biotinylated (or tagged with another similar ligand) to facilitate purification (*e.g.*, using a streptavidin column), or labeled with fluorescein or radioactive atoms to simplify detection in a binding assay. Thus, one might simultaneously prepare a collection or library of modular polymers wherein one or more sets are permanent, and other sets are biotinylated, fluorosceinated, radioactive, cleavable to acidic terminal groups, cleavable to amide terminal groups, and cleavable to neutral terminal groups.

One form of cleavable linkage was described by Geysen, WO90/09395 (incorporated herein by reference), in which the link cyclizes to a diketopiperazine moiety with concomitant cleavage of the peptide chain from the support. In the preferred embodiment, lysine having a protected α-amino group (*e.g.*, with BOC) is coupled to the support through the ε-amino group. Pro, whose carboxy terminus is esterified with a suitably reactive spacer ("X") having an orthogonally protected (*e.g.*, Fmoc) amino group is then coupled to the Lys carboxylate function. Synthesis of the modular polymer then proceeds on the amino group on deprotection:

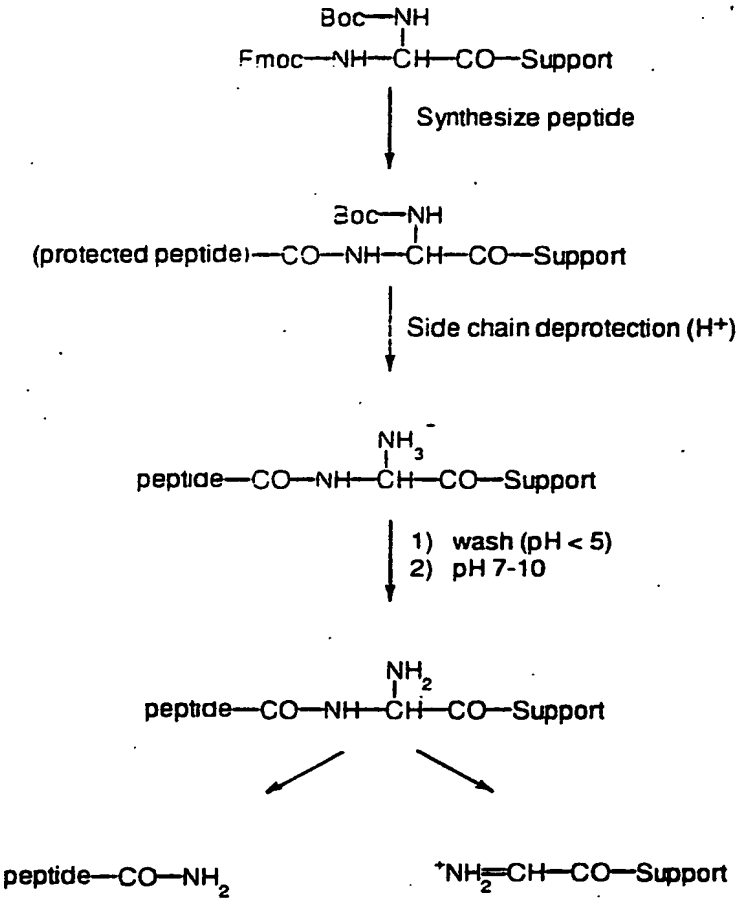


Alternatively, one may synthesize the group first and couple it to the pin:

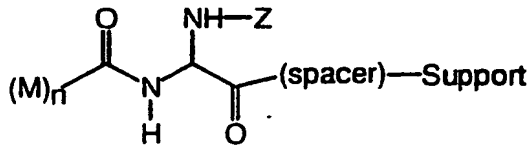


Cleavage is effected by removing the BOC (or other group) protecting the Lys α-amino group, and neutralizing the resulting $-NH_3^+$. When the Lys α-amino $-NH_3^+$ is neutralized to $-NH_2$, the amine attacks the Pro carbonyl and displaces the Pro, and with it, the modular polymer. A diketopiperazine moiety is left attached to the active surface. Alternatively, one may couple the spacing group to the active surface, to form an ester link. Coupling of a suitably protected Lys to Pro will form the same basic linker. However, this configuration leaves a diketopiperazine moiety attached to the modular polymer after cleavage from the support.

We have also developed a new linker, which facilitates cleavage of a modular polymer to provide an amide function at the point of cleavage. The modular polymer is synthesized linked to the active surface through a protected α-aminoglycine. After the modular polymer synthesis is completed, the protecting group is removed from the α-amino group. Immersing the active surface in an aqueous solution at pH 7-10 results in cleavage, leaving an amide function at the site of cleavage on the modular polymer, as shown in the Scheme below:



This linkage may be used for any type of modular polymer. The general formula for a bound modular polymer is



wherein M is a monomer, n is an integer (preferably 2-30, inclusive), and Z is a protecting group. The spacer is optional.

Other cleavable linkages may be employed. For example, there are known photocleavable linkages, which cleave upon exposure to light at a selected frequency. These linkages, and the linkages described above, are generally most suitable for use with peptide- and peptoid-based modular polymers, but may also be used with nucleic acids. Where the modular polymer is a nucleic acid, it may be conveniently removed by providing a cleavable linkage in the form of a restriction enzyme recognition site. Nucleic acid modular polymers may also include polymerase promoter/binding sites and amplification primer hybridization sites, to facilitate duplex formation and amplification (*e.g.*, by PCR).

The active surfaces may also carry identifying features to facilitate differentiation between different types of surface. For example, active surfaces of different types may have different colors and/or patterns, different sizes and/or shapes, different degrees of adherence to the pin or rod (*e.g.*, requiring a different amount of force to remove a surface from a pin), different degrees of magnetization, *etc*. Surfaces that differ in color and/or pattern may be separated by hand on inspection. Colors may be achieved by including dyes in the active surface component, either in the active surface layer or in the supporting structure underneath, or both. Patterns should generally be simple, and may be obtained, *e.g.*, by dying only half of each component. Surfaces that differ in degree of adherence may be separated from the pin or support by a measured shake or impact (removing first those surfaces that are loosely attached, followed by a more vigorous shake or impact to remove surfaces that are more tightly bound). Surfaces that differ in size and/or shape may be separated by a simple screen that allows small components to pass, while retaining larger components. Obviously, surfaces that are magnetized may be separated from non-magnetized surfaces using a magnet, electromagnet, or ferrous metal. The last two systems are more suitable to automation. Thus, one may prepare a collection or set

of modular polymers as described above, in several different forms, in one operation. The different forms may then be easily separated for further research.

The use of modular active surface components and inert components has several advantages. First, it permits one to prepare each component under optimal conditions, using the materials most suitable for the function of each component. For example, the active surface components need not display the rigidity desired in the support rods or support block: by modular construction, the rods may be made of rigid materials, while the active surface components may be made of softer materials which are optimized for use as synthesis surfaces.

Another major advantage of manufacturing the portion providing the active region as a separate entity is the minimization of cross-contamination of solutions. The polymer layer grafted onto the rods during the radiation process as described in EP 198855 is readily solvated by many solvents and as a consequence, solvents will migrate upwards through this layer by capillary action. This results in depletion of the reservoir of reagent and consequently, as described above, a larger zone of uncertain synthesis quality is created. Also, unless extreme care is taken in extensively washing these rods, the polymer layer acts as a reservoir of the solvents used in synthesis leading to the contamination of subsequent solutions. Where the portion providing the active region is manufactured separately as described herein, this migration of solvents and reagents cannot take place.

Another major advantage of manufacturing the active region as a separate entity comes about because the active region will typically be much smaller than the complete unit. Therefore, more of the active region components can be treated simultaneously to create the active region with consequent savings in materials and time.

For the purpose of illustration only, the portion providing the active region of the rod, that is, the region of the rod on which the peptide or other polymeric molecule is to be synthesized, is a cylinder with a radius of 2 mm and a height of 4 mm, the surface area of which active region is 61.8 mm^2 (assuming that only

one end of this cylinder is available for synthesis). However, if a slit 1 mm wide is made across the diameter of this portion of the rod, the surface area becomes 81.8 mm^2 (1.3 times the area of the solid cylindrical portion). The surface area of the portion providing the active region can be increased even further by modification of the shape of this portion of the rod. For instance, if eight slits, each 0.4 mm wide and 1 mm deep are made into the surface of a cylindrical portion, the surface area available for synthesis is increased to 124 mm^2 (twice the area of the unmodified cylindrical portion). It will be apparent that the surface area of the active region of the rod can be further increased by further modification of its geometric shape, and equally that such modifications can be made by molding the rod in its final desired shape or by machining a molded rod into its final desired shape.

However, in a preferred embodiment of the invention, the portion providing the active region of the rod is made by joining small particles of solid materials together, for instance, by sintering using pressure or heat or both. This can be particularly useful where it is desired to use particular harsh chemistries or corrosive solvents. For instance, glass is resistant to most solvents which would make most conventional plastic materials unstable. Thus an active region could be made by sintering together small spherical beads of glass. This could then be treated, for instance, by functionalizing the surface with an amino-silane, to make it suitable as a base on which the peptide or other polymeric compound could be synthesized. In this example, the inactive region would be made from a particularly resistant plastic such as polytetrafluoroethylene. In this way, a material such as glass, which would be an unsuitable material for the inactive region, can be used with advantage in the active region.

A further advantage of this particular embodiment of the invention is the large increase in the surface area/volume ratio of the active region can be achieved. Using the example given above, 8885 spherical rigid particles each with a radius of 0.1 mm would occupy the volume of the portion providing the active region of the rod if close packed together. The surface area of these spheres would

be 1116.4 mm^2, 17.8 times the surface area of a solid cylindrical portion. Decreasing the size of the particles to be sintered together, would provide a corresponding increase in the surface area available for synthesis. For example, decreasing the radius of the rigid spheres to be sintered to 0.055 mm increases the surface area to 2233 mm^2, about 35.5 times that of the solid cylinder. In practice, because of the process of joining the particles together, and the fact that the particles are neither uniform in size nor rigid, the theoretical increase in surface area would not be achieved. However, very significant gains in surface area available for synthesis can be achieved by making the solid support by sintering small particles of material together.

Further features of the present invention are illustrated, by way of example only, in the following example.

C. Examples

The examples presented below are provided as a further guide to the practitioner of ordinary skill in the art, and are not to be construed as limiting the invention in any way.

EXAMPLE 1

(Synthesis of Peptides)

Polyethylene pins were molded as depicted in Fig. 2, and mounted in 8 x 12 blocks as described in WO91/04266.

Removable synthesis surfaces for cleavable peptides ("cleavable crowns") were molded as depicted in Fig. 1, using polyethylene, and were then radiation-grafted as described in EP 198855 with methacrylic acid/dimethylacrylamide (10% MA, 20% DMA in methanol). Cleavable crowns were then derivatized with Boc-hexamethylenediamine according to WO91/04266 by immersing in a 60 mM solution of *t*-butoxycarbonylhexamethylenediamine (Boc-HMD), triethylamine (TEA), dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBT) (1:1:1:1.2) in

DMF. After Boc deprotection with trifluoroacetic acid, the active surface was neutralized by washing in MeOH (2 min), 5% TEA in MeOH (2 × 5 min) and MeOH (5 min). The preformed diketopiperazine linker (Boc-Lys(Fmoc)-Pro-O-HMB) was coupled at 60 mM concentration with DCC/HOBT (1:1:1.2) for a set time to obtain the desired loading (HMB = hydroxylmethylbenzoic acid). On a surface area of 130 mm^2, approximately 1.5 umoles of linker can be coupled within 90 min. Unreacted amino groups on the surface are capped by acetylation.

Removable synthesis surfaces for non-cleavable peptides ("non-cleavable crowns") were molded as depicted in Fig. 1, using polyethylene, and were then radiation-grafted as described in EP 198855 with 30% hydroxypropylmethacrylate in MeOH. The surface was then derivatized with Fmoc-Gly (30 mM), DCC and dimethylaminopyridine (DMAP) (1:1:0.2) in DMF/dichloromethane (1:3) for 15 min. The unreacted hydroxyl groups on the surface are capped by acetylation. After Fmoc deprotection in 20% piperidine/DMF and washing with DMF (2 min) and MeOH (3 × 2 min), a control Fmoc-β-Ala coupling is performed to obtain a total loading of 50 to 100 nmoles. Fmoc-β-Ala, DCC and HOBT (1:1:1.2, 30 mM) in DMF is coupled for approximately 10 min and the remaining amino groups of Gly capped by acetylation.

Each pin was loaded with four non-cleavable crowns, followed by one cleavable crown. The resulting synthesis structure was then used to synthesize a set of overlapping octapeptides derived from the *Neisseria gonorrhea* C30 strain pilin protein, amino acids 30-52 (RAQVSEAILLAEGQKSAVYEYYLNHGKWP). Thus, any given pin carried five crowns having identical octapeptides, with each pin carrying an octapeptide different from the octapeptides carried on other pins.

EXAMPLE 2

(Assay of Peptides)

Assay of Non-Cleavable Peptides:

The non-cleavable crowns were removed from the 5-position pins and were placed in corresponding positions on blocks holding 1-position pins (see WO91/

04266) to provide four identical epitope libraries. Each block was tested for antibody binding by ELISA using a 1/20,000 dilution of a rabbit anti-pillin antiserum. The results (shown in Fig. 3) demonstrate that all four sets of peptides are essentially identical.

Assay of Cleavable Peptides:

Cleavable crowns were then removed from the pins, and the peptides removed. Each crown was treated with 800 µL of cleavage solution (0.1 M NH_4HCO_3, 40% CH_3CN, pH 8.4). Aliquots were submitted to amino acid analysis. HPLC demonstrated high levels of purity. The typical yield of peptide was about 600 nmole/crown.

Each peptide was then used at three concentrations to illustrate solution-phase competition for binding of the antibody to the non-cleaved peptide set. Four serum preparations were tested:

1) 1/21,000 serum dilution, with no added peptide (Fig. 4A);
2) 1/21,000 serum dilution, peptide added to 35 nmole/mL (Fig. 4B);
3) 1/21,000 serum dilution, peptide added to 3.5 nmole/mL (Fig. 4C);
4) 1/21,000 serum dilution, peptide added to 0.35 nmole/mL (Fig. 4D).

The antiserum and peptides were mixed and incubated for 1 hr at room temperature before testing with the set of bound (noncleavable) peptides. The ELISA results are shown in Fig. 4: a concentration of 35 nmole/mL of the cleaved peptides (Fig. 4B) was sufficient to inhibit antibody binding to the noncleavable peptides essentially completely.

What is Claimed:

1. A synthesis support for use in the synthesis of modular polymers, said synthesis support comprising:

a plurality of generally annular synthesis components, comprising an active surface adapted to support solid-phase synthesis of a modular polymer; and

an elongate, inert support rod, positioned axially to said generally annular synthesis components, wherein said support rod retains said generally annular synthesis components in a collinear configuration.

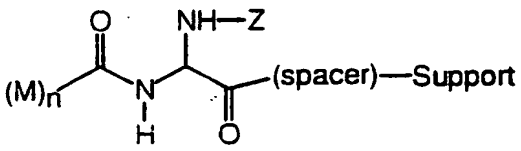
2. The synthesis support of claim 1, wherein said inert support rod is designed to receive about 5 generally annular synthesis components.

3. The synthesis support of claim 1, wherein said inert support comprises polyethylene or polypropylene.

4. The synthesis support of claim 1, wherein a plurality of synthesis supports are fixed in an array.

5. The synthesis support of claim 4, wherein said array is an 8 × 12 array.

6. A bound modular polymer of the formula:



wherein each M is independently a monomer;
n is an integer from 2 to 100; and
Z is a protecting group.

7. The bound modular polymer of claim 6, wherein each M is an amino acid.

8. The bound modular polymer of claim 7, wherein n is an integer from 2 to 20.

9. The bound modular polymer of claim 8, wherein n is an integer from 2 to 10.

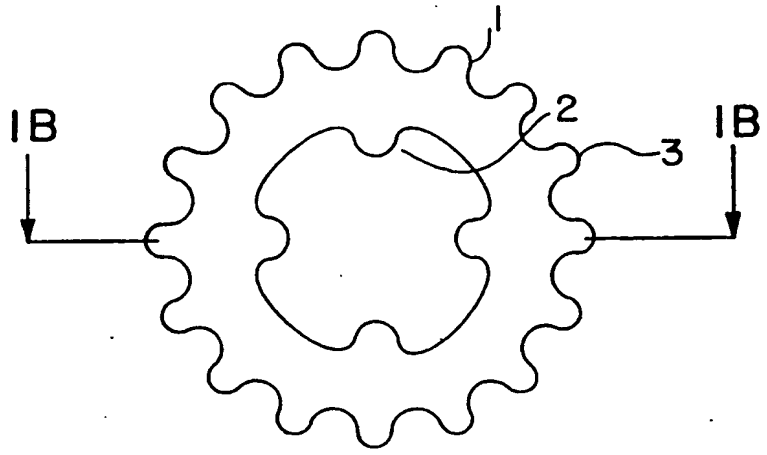


FIG. 1(A)

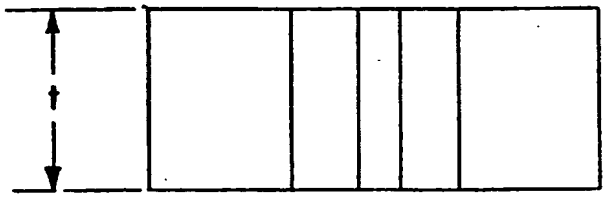


FIG. 1(B)

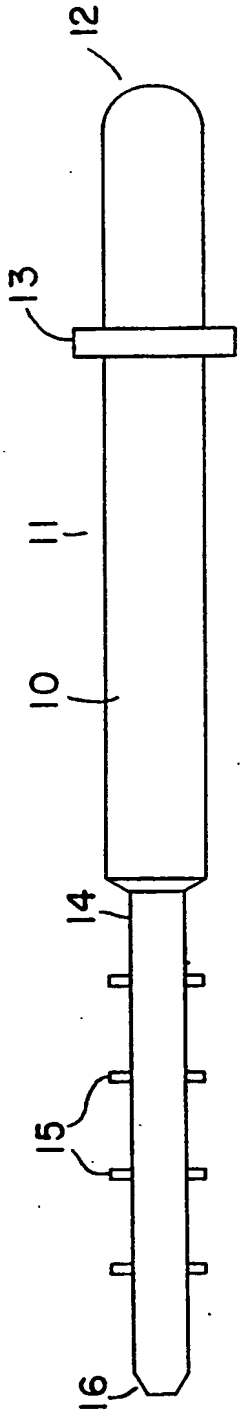


FIG. 2(A)

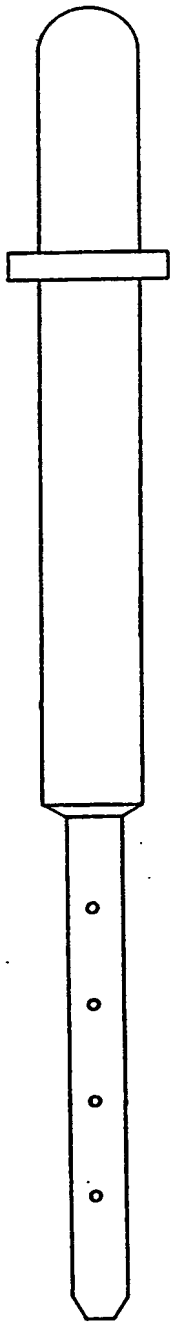


FIG. 2(B)

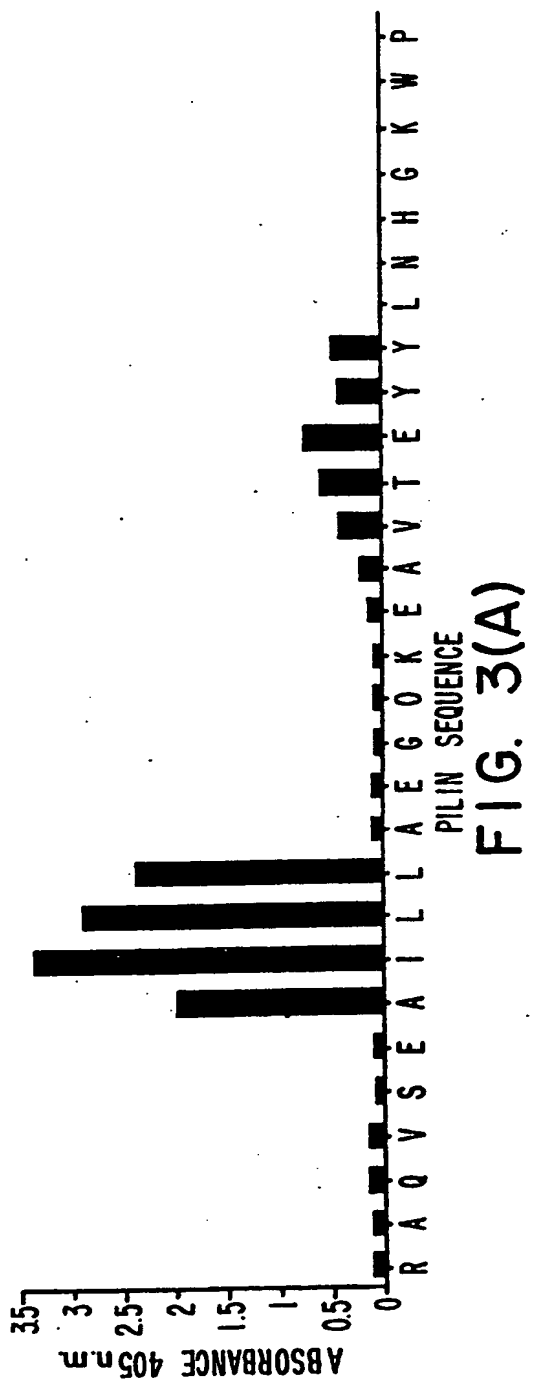

ABSORBANCE 405 n.m
0
0.5
1
1.5
2
2.5
3
3.5
R A Q V S E A I L L A E G Q K E A V T E Y Y L N H G K W P
PILIN SEQUENCE

FIG. 3(A)

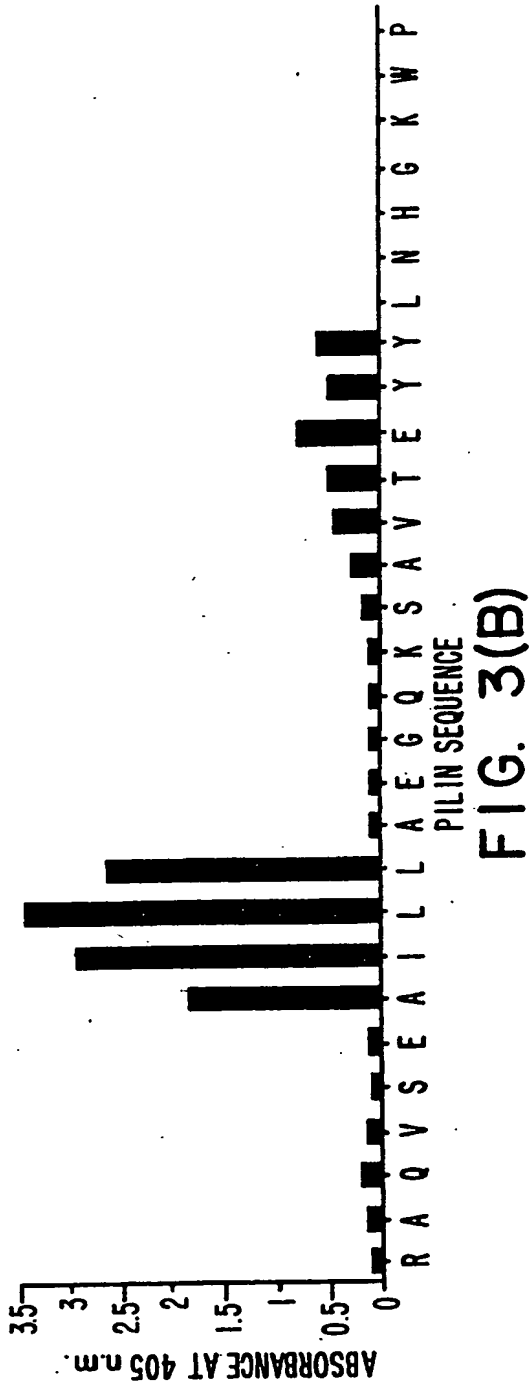

ABSORBANCE AT 405 n.m
0
0.5
1
1.5
2
2.5
3
3.5
R A Q V S E A I L L A E G Q K S A V T E Y Y L N H G K W P
PILIN SEQUENCE

FIG. 3(B)

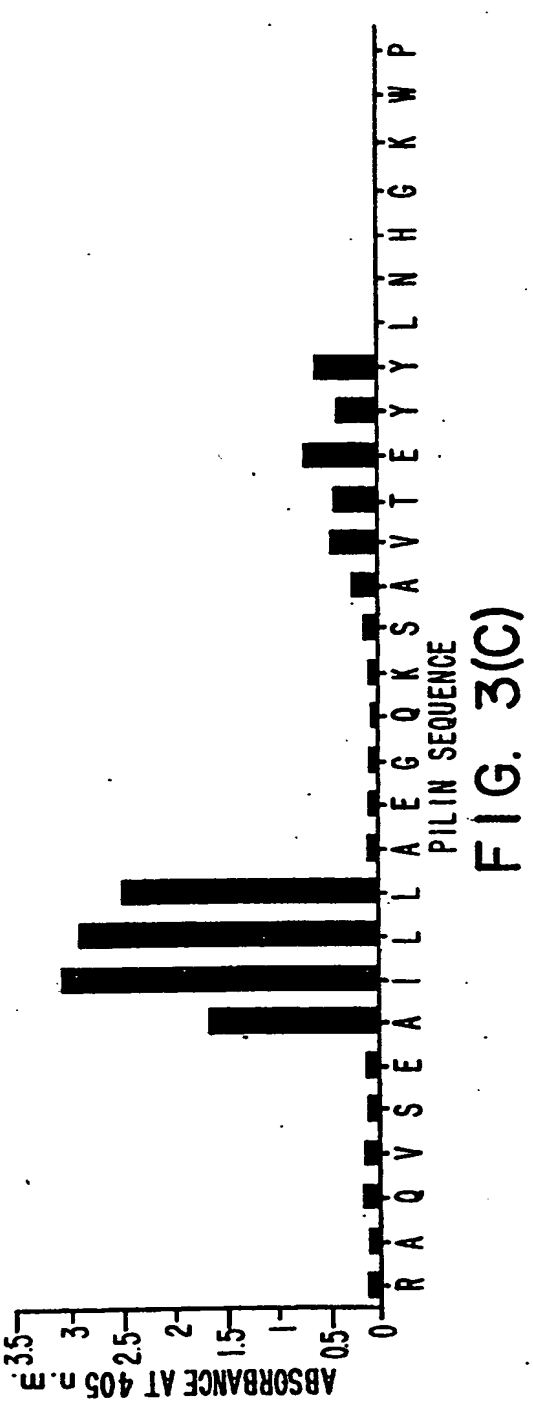

ABSORBANCE AT 405 n.m.
0
0.5
1
1.5
2
2.5
3
3.5
R A Q V S E A I L L A E G Q K S A V T E Y Y L N H G K W P
PILIN SEQUENCE

FIG. 3(C)

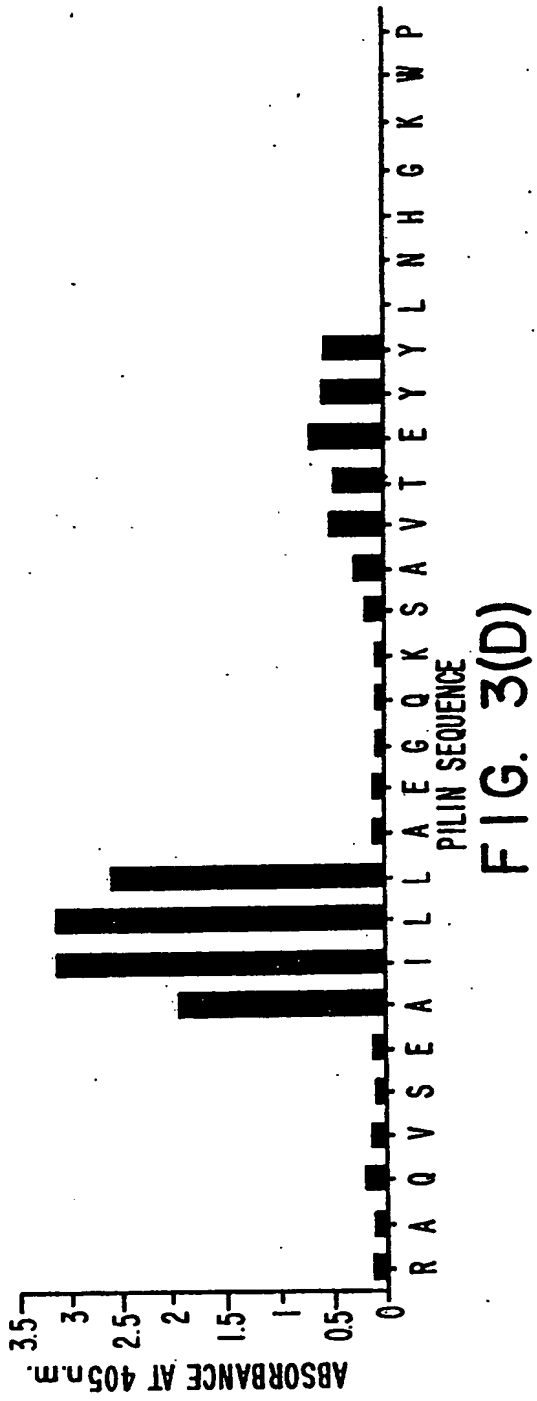

ABSORBANCE AT 405n.m.
0
0.5
1
1.5
2
2.5
3
3.5
R A Q V S E A I L L A E G Q K S A V T E Y Y L N H G K W P
PILIN SEQUENCE

FIG. 3(D)

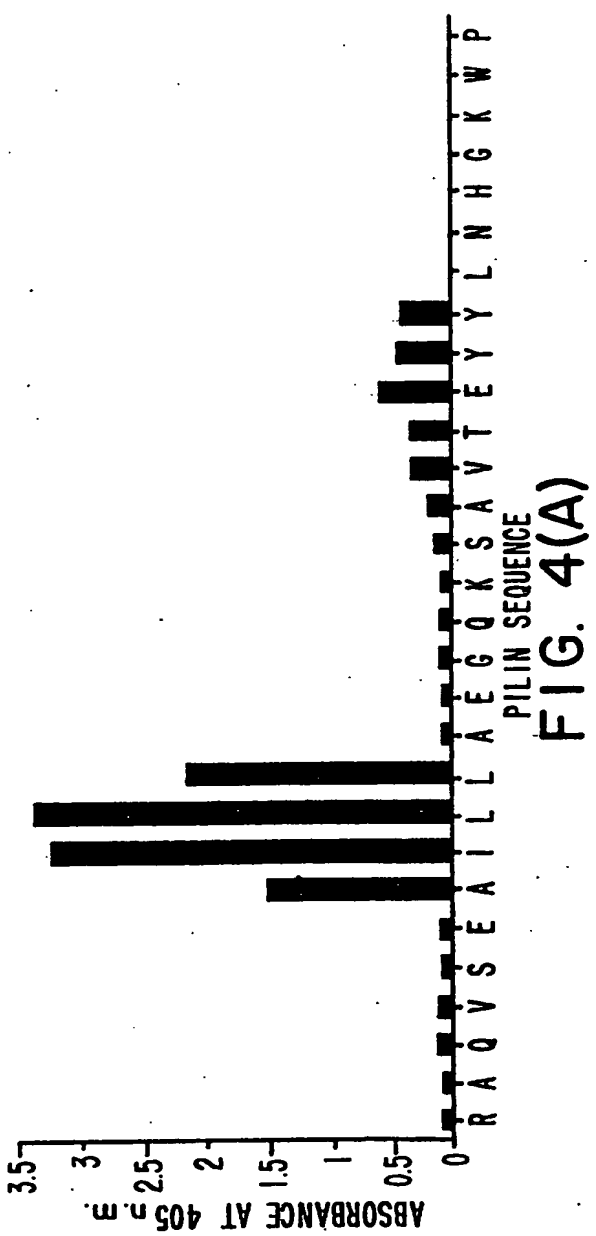

ABSORBANCE AT 405 n.m.
3.5
3
2.5
2
1.5
1
0.5
0
R A Q V S E A I L L A E G Q K S A V T E Y Y L N H G K W P
PILIN SEQUENCE

FIG. 4(A)

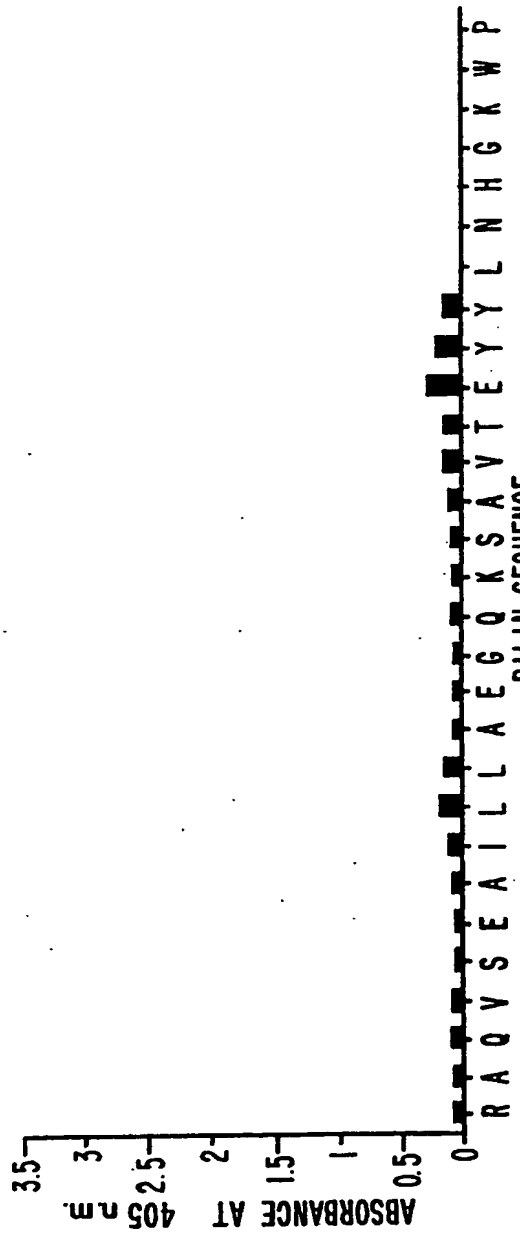

ABSORBANCE AT 405 n.m.
3.5
3
2.5
2
1.5
1
0.5
0
R A Q V S E A I L L A E G Q K S A V T E Y Y L N H G K W P
PILIN SEQUENCE

FIG. 4(B)

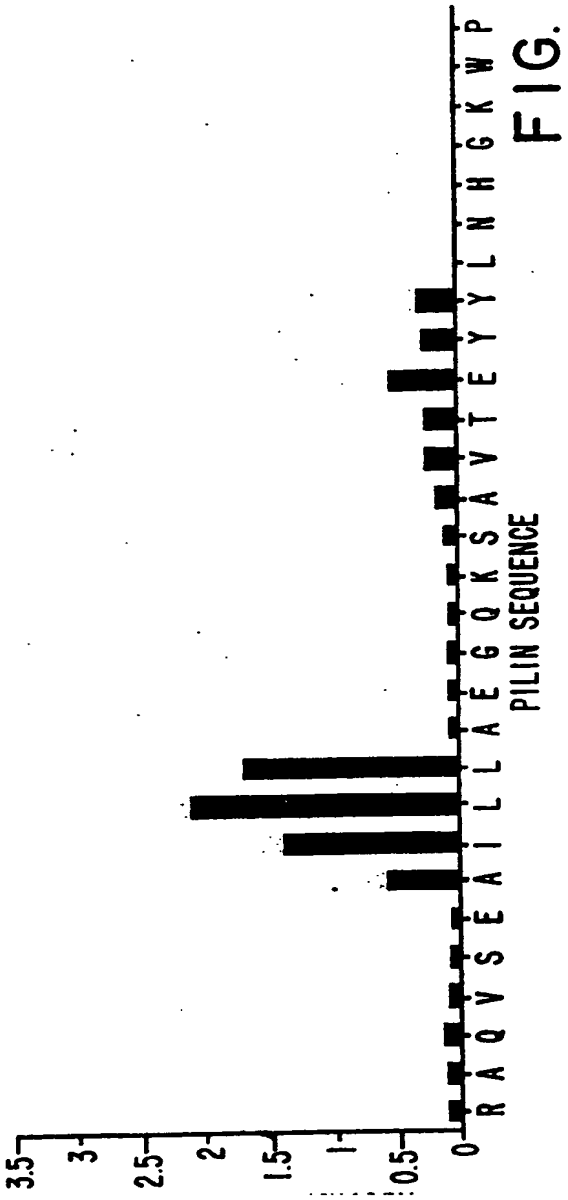

3.5
3
2.5
2
1.5
1
0.5
0
R A Q V S E A I L L A E G Q K S A V T E Y Y L N H G K W P
PILIN SEQUENCE

FIG. 4(C)

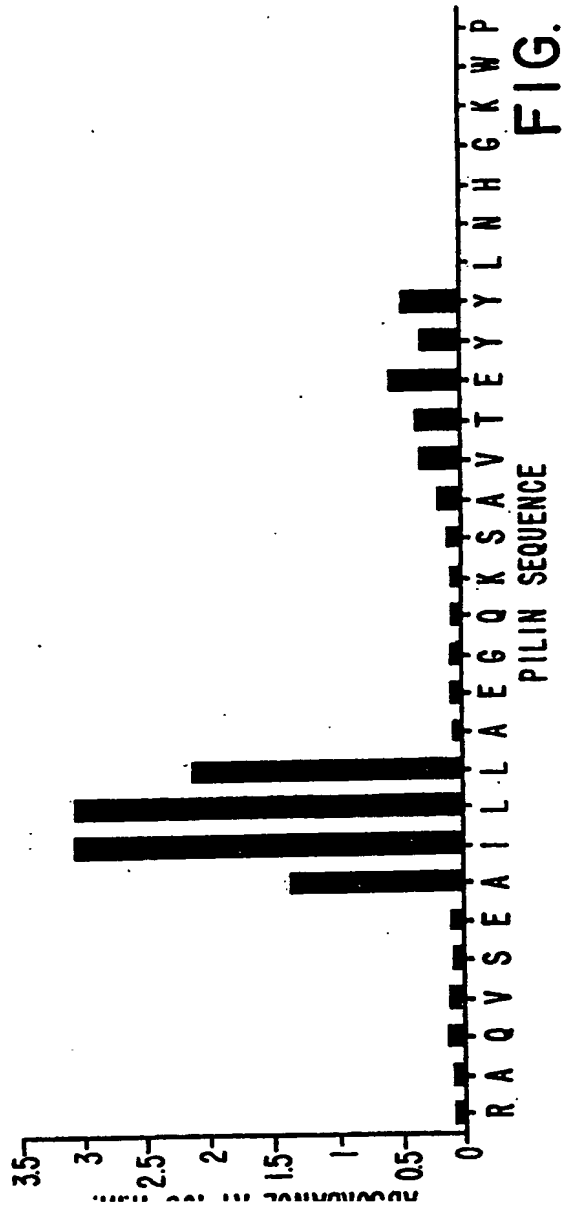

3.5
3
2.5
2
1.5
1
0.5
0
R A Q V S E A I L L A E G Q K S A V T E Y Y L N H G K W P
PILIN SEQUENCE

FIG. 4(D)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 93/00546

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.[5] C07K 1/04, C07K 17/06, C07K 17/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: FILE WPAT: see below

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

AU: IPC: C07K 17/00, C07K 17/02, C07K 17/06, C07K 17/14

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)

FILE WPAT (SYNTHE: & SUPPORT:) & (C07K or C07H)
FILE CASA (PROTEIN & PEPTIDE & POLYPEPTIDE & SOLID PHASE) & (SUPPORT/CARRIER)
FILE CASM (SYNTH: & SUPPORT:) & (POLYMER/PEPTIDE:/OLIGONUC:)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X,Y Y	AU-A-63593/90 (COSELCO MIMOTOPES P/L) 4 April 1991 (04.04.91) See page 4 line 13, page 10 lines 25-31, Example 1	1,3,4,5 2
Y	AU-B-49869/90 (642403) (COSELCO MIMOTOPES P/L) 23 August 1990 (23.08.90) See Example 4	1-5
Y	AU-B-25429/84 (576750) (COMMONWEALTH SERUM LABORATORIES COMMISSION) 14 November 1985 (14.11.85) See page 7 lines 8-13	1-5

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 January 1994 (13.01.94)	25 JAN 1994 (25.01.94)

Name and mailing address of the ISA/AU

AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION
PO BOX 200
WODEN ACT 2606
AUSTRALIA

Facsimile No. (06) 2853929

Authorized officer

M. ROSS

Telephone No. (06) 2832295

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
A	AU-B-12260/88 (602753) (BAYLOR COLLEGE OF MEDICINE) 27 August 1988 (27.08.88)	
A	AU-B-48561/85 (558709) (F. HOFFMAN-LA ROCHE & CO AG) 24 April 1986 (24.04.86)	
A	AU-A-76456/87(STEE, S.L.) 22 September 1988 (22.09.88)	
A	US,A,4515920 (THE ROCKEFELLER UNIVERSITY) 7 May 1985 (07.05.85)	
A	WO,A,84/02526 (SOUTHERN ILLINOIS UNIVERSITY FOUNDATION) 5 July 1984 (05.07.84)	
A	DE,A,3935572 (GESELLSCHAFT FÜR BIOTECHNOLOGISCHE FORSCHUNG mbH) 2 May 1991 (02.05.91)	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 93/00546

Box I **Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)**

This international search report has not established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Claims 1 to 5 define apparatus for use in the synthesis of modular polymers. Claims 6 to 9 define a polymer attached to a support by chemical linking and spacing groups. Neither set of claims are limited to the necessary use of the subject of the other claims.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically

claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member					
AU-A 63593/90	EP	498801	WO	91/04266		
AU-B 49869/90	CA	2047191	EP	458841	NO,A	913186
	NZ	232562	WO	90/09395	JP	4504416
AU-B 25429/84	AT	69890	CA	1220420	DE	3485299
	DK,A	5322/84	EP	138855	JP	60500684
	NO,A	844296	NZ	207394	WO	84/03564

END OF ANNEX